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Fibrocytes in Scleroderma Lung Fibrosis

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1. Introduction

Systemic sclerosis (SSc) or “Scleroderma” is a disease characterized by cutaneous and visceral fibrosis that affects both the skin and internal organs. The worldwide prevalence ranges from 50-300 cases per million (Chiffot et al., 2008) and much of the morbidity in this population results from pulmonary complications. In fact, nearly 70% of scleroderma patients show some form of lung disease. Of the two forms of lung involvement, pulmonary arterial hypertension and interstitial lung disease, the latter has emerged as the greatest cause of death in these patients. The lungs of patients with scleroderma associated interstitial lung disease (SSc-ILD) exhibit replacement of the normal lung architecture with inflamed and fibrotic tissue that cannot participate in gas exchange. While approximately 42% of patients with SSc-ILD die of disease progression within ten years of diagnosis (Steen & Medsger, 2007) evidence is emerging that some patients progress slowly and in some cases spontaneously improve while others follow an accelerated clinical course (Goh et al., 2008). There is currently no way to predict which patients will progress rapidly and require more intensive therapy (Daoussis et al., 2010 ; Swigris et al., 2006; Tashkin et al., 2006) and/or referral for lung transplantation (D'Ovidio et al., 2005a; D'Ovidio et al., 2005b); and which patients will follow a more indolent course requiring less intense follow up. Therefore the development of a clinically predictive measure of pathologic progression would benefit physicians caring for patients with this disease. The ideal biomarker would be present in easily accessible clinical specimens, would be a potential contributor to disease development, and would be easily studied in murine models of disease. For this reason, peripheral blood fibrocytes have emerged as an exciting new area of study in the field of Scleroderma (Gan et al., 2011; Mathai et al., 2010; Peng et al., 2011 ; Tourkina et al., 2011; Reilkoff et al., 2011).

2. Fibrocytes

Fibrocytes are blood borne collagen-producing cells that were initially described in 1994. Since then, they have been associated with a broad range of fibrosing disorders including autoimmune illnesses and chronic inflammatory diseases. In some of these diseases, high circulating levels correlate with poor outcomes. In addition to extracellular matrix (ECM) synthesis, fibrocytes display other functions including antigen presentation and the secretion of pro-fibrotic and pro-angiogenic factors.

2.1 Disease associations

Identified by their co-expression of leukocyte markers such as CD45, extracellular matrix proteins such as Collagen-1 α and in some cases markers expressed by progenitor cells such as CD34 (Bucala et al., 1994), fibrocytes are easily detected via flow cytometric and in vitro culture techniques. These approaches demonstrate that abnormalities in peripheral blood fibrocytes exist in diverse forms of autoimmune disease such as rheumatoid arthritis (Galligan et al., 2010), autoimmune thyroiditis (Douglas et al., 2009), amyopathic antisynthetase syndrome (Peng et al., 2011), and scleroderma (Gan et al., 2011 ; Mathai et al., 2010). Elevations in peripheral blood fibrocytes are seen in chronic inflammatory disorders not traditionally associated with autoimmunity such as idiopathic pulmonary fibrosis (Mehrad et al., 2007; Mehrad et al., 2009; Moeller et al., 2009), asthma (Schmidt et al., 2003; Nihlberg et al., 2006; Wang et al., 2008), nephrogenic systemic fibrosis (Vakil et al., 2009), cardiovascular disease (Falk, 2006), pulmonary hypertension (Nikam et al., 2011), and even normal aging (Mathai et al., 2010). Thus it is not surprising that the role fibrocytes play in tissue repair and remodeling is a developing area of interest in the study of fibrosis and autoimmunity.

2.2 Identification of fibrocytes in the circulation

Flow cytometry identifies fibrocytes from the circulation or tissue using the combination of characteristic cell surface marker expression with intracellular staining for collagens or extracellular matrix components. Human fibrocytes express hematopoietic markers such as CD45 (Bucala et al., 1994), Leukocyte specific protein-1 (Yang et al., 2002), as well as markers of adhesion and motility (Pilling et al., 2009), chemokine receptors such as CXCR4 (Mehrad et al., 2007), proteins important in host defense and scavenger receptors (Pilling et al., 2009), antigen presentation (Chesney et al., 1997), and cell surface enzymes such as CD10 and CD13 (Pilling et al., 2009). Fibrocytes typically lack markers of lymphocytes (Bellini & Mattoli, 2007; Pilling et al., 2009). Circulating and cultured fibrocytes also express CD34 (Bucala et al., 1994), a motility protein that allows fibrocytes to be distinguished from other collagen-containing cell types such as fibroblasts and macrophages (Reilkoff et al., 2011). However, because CD34 is frequently lost upon entry into target tissue (Peng et al., 2011; Phillips et al., 2004) its absence does not rule out a cell as being a fibrocyte. Fibrocytes also produce a wide array of ECM components (Bianchetti et al., 2011; Bellini & Mattoli, 2007; Pilling et al., 2009). A listing of fibrocyte markers is shown in Table 1.

2.3 Differentiation and homing

Insight into potential fibrocyte functions may be gleaned from an understanding of the factors promoting their differentiation and recruitment. Fibrocytes differentiate from a precursor population within the CD14⁺ monocyte fraction of peripheral blood (Abe et al., 2001). The monocyte to fibrocyte transition, which is increased by enrichment for CD11b(+) CD115(+) Gr1(+) expressing monocytes, is promoted by direct contact with activated CD4⁺ lymphocytes via an mTOR-PI3 kinase dependent pathway (Niedermeier et al., 2009). Other studies have determined that the fibrocyte precursor expresses components of the Fc γ receptor (Pilling et al., 2003). Inhibition of this receptor with the short pentraxin protein serum Amyloid P reduces fibrocyte outgrowth in human (Pilling et al., 2003; Pilling et al., 2006) and rodent samples (Murray et al., 2011; Pilling et al., 2007). This effect appears to be

mediated via an ITIM-dependent mechanism (Castano et al., 2009). The monocyte to fibrocyte transition is inhibited by exposure to TH1 cytokines (IFN γ , TNF, and IL-12); and is augmented by TH2 cytokines (IL-4 and IL-13) (Shao et al., 2008). Fibrocyte differentiation is further stimulated by TGF- β 1, and via engagement of the β 1 integrin subunit (Bianchetti et al., 2011 ; Gan et al., 2011 ; Nikam et al., 2011).

Murine modeling demonstrates that certain chemokine receptors such as CCR2, CCR7, and CXCR4 promote fibrocyte recruitment to diseased tissue (Phillips et al., 2004; Moore et al., 2006; Sakai et al., 2006). Thus it is particularly relevant that human fibrocytes express the chemokine receptors CCR3 (eotaxin receptor) and CCR5 (MCP-1 receptor). Human fibrocytes also express Semaphorin 7a (Quan et al., 2004), a GPI-anchored membrane protein with important immunomodulatory effects (Czopik et al., 2006; Suzuki et al., 2007). Our own work in scleroderma patients demonstrates an association between fibrocytes serum concentrations of soluble factors such as TNF, IL-10, MCP-1 and IL-1 receptor antagonist (IL-1Ra), suggesting that fibrocytes may be mobilized into the circulation in response to one or more of these factors. Similarly, idiopathic pulmonary fibrosis (IPF) patients demonstrate high levels of CXCL12 in their blood and lungs, which is the cognate ligand for CXCR4, and these levels correlate with circulating fibrocyte concentrations (Mehrad et al., 2007). When viewed in combination, this array of stimulatory factors suggests that fibrocytes are recruited to injured tissue where they may play a role in the healing processes by via both immunomodulatory and ECM-producing effects.

2.4 Immunologic function

One school of thought posits that the ultimate phenotype of fibrocytes is the activated myofibroblast (Abe et al., 2001; Phillips et al., 2004; Quan et al., 2004; Gomperts & Strieter, 2007). This hypothesis is based on several studies demonstrating cultured fibrocytes respond to TGF- β 1 by expressing α -SMA and contracting collagen gels *in vitro* (Abe et al., 2001; Phillips et al., 2004; Quan et al., 2004; Gomperts & Strieter, 2007). However, because *in vivo* studies using bone marrow chimeras show only minimal contributions of fibrocytes to α -SMA production in some models (Hashimoto et al., 2004; Kisseleva et al., 2006; Lin et al., 2008), this feature of fibrocytes may not dominate in tissue remodeling responses. When viewed in this light, it is particularly important to examine the immunomodulatory functions that fibrocytes are known to possess.

Type of Marker	Level of Expression	Reference
<i>Adhesion and Motility</i>		
CD9, CD11a, CD11b, CD11c, CD43, CD164, Mac2, LSP-1	Moderate	(Galligan et al., 2011; Bucala et al., 1994; Yang et al., 2002; Pilling et al., 2009)
CD34	Low	(Mathai et al., 2010; Bucala et al., 1994; Barth et al., 2002)
CD29, CD44, CD81, ICAM-1, CD49 complex, CD81	Low	(Mathai et al., 2010; Nikam et al., 2011; Bucala et al., 1994; Pilling et al., 2009)

Type of Marker	Level of Expression	Reference
<i>Cell Surface Enzymes</i>		
CD10, CD172a	Low	(Bucala et al., 1994)
CD13, Prolyl-4-hydroxylase	Low	
FAP	Low	
<i>Scavenging receptors and host defense</i>		
CD14, CD68, CD163, CD206, CD209, CD35, CD36	Variable	(Bucala et al., 1994)
<i>Fcy receptors</i>		
CD16, CD32a, CD32b, CD32c	Moderate	(Bucala et al., 1994)
<i>Chemokine receptors</i>		
CCR2, CCR5, CCR4, CCR7, CCR9, CXCR1, CXCR4, CXCR3R1	Moderate	(Mehrad et al., 2007; Pilling et al., 2009)
<i>Antigen Presentation</i>		
CD80, CD86, MHCI, MCHII	Low	(Chesney et al., 1997)
<i>Extracellular matrix</i>		
Collagen-I/III/IV, vimentin, tenascin	Low	(Bucala et al., 1994; Schmidt et al., 2003; Pilling et al., 2009)
Fibronectin, α-SMA	Variable	(Bianchetti et al., 2011; Bucala et al., 1994; Schmidt et al., 2003; Pilling et al., 2009)
Collagen V	Moderate	(Bianchetti et al., 2011)
<i>Glycosaminoglycans</i>		
Perlecan, Veriscan, Hyaluronan	Moderate	(Bianchetti et al., 2011)
Decorin	Low	
<i>Miscellaneous</i>		
Semaphorin 7a	Low	(Mathai et al., 2010)
CD115	None	(Pilling et al., 2009)
Thy1.1	Low	(Douglas et al., 2009)
CD105	Low	(Pilling et al., 2009)

Table 1. Fibrocyte Marker Expression

For example, human fibrocytes respond to Interleukin-1 beta (IL-1β) by increasing secretion of proinflammatory mediators such as Interleukin-6 (IL-6), Interleukin-8 (IL-8), and Chemokine (C-C motif) ligand 21. Porcine fibrocytes respond to innate immune stimulation adopting certain properties of antigen presenting cells via their expression of Major Histocompatibility Complex I and II, CD80 and CD86 (Balmelli et al., 2007). This function is also seen in human fibrocytes (Chesney et al., 1997). In addition to these pro-inflammatory effects, fibrocytes also secrete paracrine factors such as Interleukin-10 (Chesney et al., 1998),

TGF- β 1, and platelet-derived growth factor (PDGF), which are expected to dampen inflammation and induce repair and angiogenesis.

This latter function is augmented via their secretion of matrix metalloproteinases (MMPs), vascular endothelial growth factor (VEGF), PDGF-A, hepatocyte growth factor (HGF), granulocyte-macrophage colony stimulating factor (GM-CSF), basic fibroblast growth factor (b-FGF), IL-8 and IL-1 β (Hartlapp et al., 2001). Via their expression of Semaphorin 7a, fibrocytes may directly activate macrophages and dendritic cells (Suzuki et al., 2007) and negatively regulate T cell responses (Czopik et al., 2006). This array of functions suggest that fibrocytes are a highly plastic cell population that may significantly promote the aberrant immune response and tissue remodeling seen in scleroderma. In order to further explore the factors promoting fibrocyte accumulation in the blood and lungs of patients with SSc-ILD, we performed the following set of translational studies.

3. Materials and methods

TGF β 1 transgenic mice: All mouse experiments were approved by the Yale School of Medicine Institutional Animal Care and Use Committee. The CC10-tTS-rtTA- TGF- β 1 transgenic mice used in this study have been described (Lee et al., 2004). The Sema-7a null mice were provided by Dr. Alex Kolodkin (Johns Hopkins) and have been described previously (Pasterkamp et al., 2003).

Doxycycline Administration: Eight-to-10 week old CC10-tTS-rtTA- TGF- β 1 transgene positive (Tg+) or transgene negative (Tg-) mice with the Sema-7a locus null or intact were given doxycycline 0.5mg/ml in their drinking water for up to 2 weeks.

Bone marrow transplantation: Mice were prepared for bone marrow transplantation using 400 cGy total body irradiation. Bone marrow harvest, preparation, and injection were performed as previously described (Herzog et al., 2006).

β 1 integrin blocking antibodies: TGF- β 1 Tg+ and Tg- mice with an intact Sema-7a locus were injected with 125 μ g of a neutralizing anti- β 1 integrin antibody or isotype control (both from Biolegend) as previously described (Bungartz et al., 2006).

Lung inflammation: Euthanasia and bronchoalveolar lavage were performed as previously described (Lee et al., 2004). Lung inflammation was assessed via bronchoalveolar lavage (BAL) samples as described previously (Lee et al., 2004).

Collagen assessment: Total left lung collagen was measured using the Sircol Assay following the manufacturer's protocol (Biocolour, Ireland).

Flow cytometry for fibrocytes: Flow cytometry was performed as previously described (Mathai et al., 2010).

Histologic analysis: Formalin-fixed and paraffin-embedded lung sections were stained with hematoxylin and eosin to assess gross morphology or Mallory's trichrome stains to visualize collagen deposition.

mRNA analyses: Total RNA was obtained using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Primers specific for human Sema-7a, β 1 integrin subunit, Plexin C1 and GAPDH, and murine β 1 integrin subunit, Plexin C1, and β -actin were purchased from Superarray Bioscience. Gene expression levels were quantified using real time RT-PCR (Applied Biosystems), according to the manufacturer's protocols and normalized to GAPDH or β -actin mRNA.

Human cell isolation and culture: All studies were performed with HIC approval and written informed consent at Yale University School of Medicine. Cells were cultured as previously described (Mathai et al., 2010).

TUNEL: TUNEL was performed as previously described (Murray et al., 2011).

Caspase activation: Detection of caspase cleavage and activation using immunohistochemistry was performed as previously described (Lee et al., 2004).

Annexin V: Flow cytometric assessment of annexin V externalization was performed via flow cytometry as previously described (Lee et al., 2004).

Statistics: Normally distributed data were expressed as means \pm SEM and assessed for significance by Student's *t* test or ANOVA as appropriate. Data that were not normally distributed were assessed for significance using the Mann-Whitney U test.

4. Results

4.1 Circulating fibrocytes are elevated in SSc-ILD

In order to determine whether fibrocytes are found with increased frequency in the circulation of patients with SSc-ILD, peripheral blood mononuclear cells were obtained from a cohort of patients with SSc-ILD ($n=12$) and normal controls ($n=27$) and assessed for fibrocytes based on the coexpression of CD45 and Pro-Collagen-I α . This double positive population was seen in both cohorts, and while overall percentages of fibrocytes were not increased in the SSc-ILD subjects, total quantities of collagen producing leukocytes, were increased by 79% in the SSc-ILD cohort compared to controls (Figure 1, $p<0.05$) (Mathai et al., 2010) and reflected impairments in ventilatory function as measured by the percent-predicted forced vital capacity (FVC) (E. Herzog, unpublished data). These cells displayed enriched expression of CD34 and in some cases also expressed CD14, indicating both their multipotent potential as well as their monocytic origin. Since this work was first published these results have been confirmed by at least one other group (Tourkina et al., 2011) indicating the reproducibility of the fibrocyte assay in this patient population.

4.2 Circulating fibrocytes exist in a profibrotic milieu

In order to determine the immunologic milieu in which these fibrocytes exist, further phenotyping of these subjects was performed. Further analysis of circulating monocytes from these individuals found them to be skewed towards an alternatively activated, profibrotic phenotype as evidenced by a propensity to adopt both CD163 expression and CCL18 secretion when stimulated with LPS. Multianalyte ELISA of plasma from these patients demonstrated increased concentrations of profibrotic cytokines and chemokines such as IL10, TNF- α , and IL-1 RA (Mathai et al., 2010). Many of the mediators are commonly associated with alternatively activated macrophages (or “M2”) leading to some speculation that fibrocytes may simply represent an intermediate population in the terminal differentiation of profibrotic macrophages (Reilkoff et al., 2011). Another interesting aspect of these data were that similar (though not identical) results were seen in a cohort of aged but otherwise healthy individuals, leading to the speculation that the presence of fibrocytes may represent a previously unrecognized form of immunosenescence in patients with SSc-ILD. The presence of increased fibrocytes in the senescence associated mouse model of accelerated aging supports this hypothesis though further work is required to prove its validity.

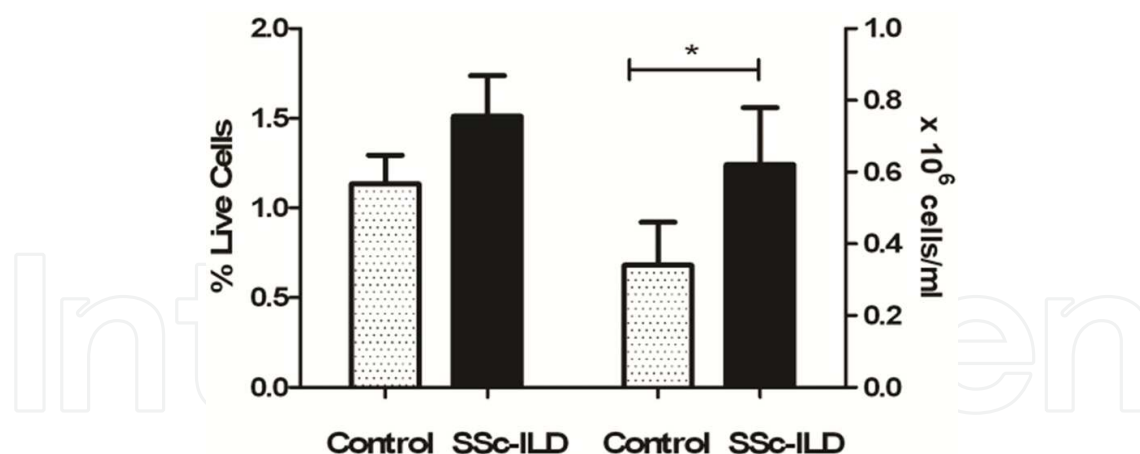


Fig. 1. Comparison of CD45+/pro-Col-I α + percentages (left axis) and quantities (right axis) in normal (n=27, gray bar) and SSc-ILD subjects (n=12, black bar). CD45+/pro-Col-I α + cell quantities, but not percentages, are increased in SSc-ILD. (Mathai et al., 2010)

4.3 Local apoptosis regulates fibrocyte accumulation

While it has long been noted that fibrocytes arise in the setting of profibrotic stimuli such as TGF- β 1, the local factors regulating the monocyte to fibrocyte transition have remained largely unexplored. Several of our recent studies, however, lend insight into these processes. One outstanding question in this area has been the relationship between injury and fibrocytes. A requirement for apoptosis in the intrapulmonary accumulation of fibrocytes was recently explored in a model of lung fibrosis caused by inducible, lung-specific overexpression of the bioactive form of the human TGF- β 1 gene. These mice develop an epithelial injury response dominated by apoptosis that peaks at 48 hours following doxycycline administration. Shortly thereafter a dense monocytic infiltrate arises that is predominantly composed of CD206+ M2 macrophages (Murray et al., 2011). These macrophages persist, and are required for the induction of activated myofibroblast development and the induction of a robust accumulation of ECM products and fibrosis that is evident by between 10-14 days of doxycycline exposure. It is not until fibrosis is relatively well established that intrapulmonary fibrocytes appear in this model. Because scleroderma related lung disease is characterized by heightened responsiveness to autocrine TGF- β 1 signaling via both canonical and non-canonical pathways (Sargent et al., 2011), this model is an ideal tool to study the pathogenesis of SSc-ILD.

The role of apoptosis in fibrocyte accumulation was tested in this model using a fairly straightforward approach. TGF- β 1 mice were randomized to receive systemic injections of the pan-caspase inhibitor Z-VAD/fmk or vehicle control. Reductions in apoptosis were confirmed at 48 hours by comparison of TUNEL staining and caspase 3 activation in Z-VAD/fmk treated TGF- β 1 mice. Reductions in lung inflammation and fibrosis were noted at day 14. Fibrocytes, defined via classical flow cytometric criteria by CD45 and Pro-Collagen-I α co-expression, were reduced by nearly 10-fold at the 14 day time point (Figure 2a, $p < 0.001$). The human relevance of these findings was supported by the finding that SSc-ILD lungs contain increased levels of CD45+ collagen-producing cells (Peng et al., 2011), and that these lungs contain increased numbers of TUNEL+ve apoptotic cells. Additionally CD14+ monocytes cultured in the presence of Z/VAD-fmk fail to adopt the spindle-shaped, collagen expressing phenotype that characterizes fibrocytes (Figure 2c,d, $p < 0.001$). Thus, from these studies it is reasonable to conclude that fibrocytes arise in response to apoptotic stimuli.

In addition to the information regarding the precise relationship between apoptosis and fibrocytes, these studies also allowed new insight into the relationship between M2 macrophages and fibrocytes. Recent literature has suggested that macrophages are capable of expressing collagen (Pilling et al., 2009) and thus the CD45/Col-I α signature may not sufficiently differentiate between fibrocytes and alternatively activated (M2) macrophages. Prior studies have demonstrated that alveolar macrophages obtained from patients with SSc-ILD demonstrate a distinct M2 profibrotic phenotype and that alternatively activated macrophages (M2) in part regulate fibrosis (Atamas et al., 2003). Thus it could be argued that the reduction in CD45/Col-I α cells previously noted could be related to changes in alternatively activated macrophages quantities or rather that M2 macrophages regulate the appearance of CD45/Col-I α cells. However, in our studies caspase inhibition demonstrated no significant differences in numbers of CD206/MRC positive cells between sham treated and ZVAD treated mice, nor was there a significant difference in M2 related genes CD206/MRC and MSR-1 using quantitative RT-PCR between the two cohorts (Peng et al., 2011). Likewise, in a separate set of studies in which M2 macrophages were removed by intratracheal instillation of liposomal clodronate, removal of M2 macrophages did not affect intrapulmonary fibrocyte content. Curiously, fibrocytes persisted despite profound attenuation of collagen deposition (Murray et al., 2011). When viewed in combination, these studies suggest that fibrocytes and M2 macrophages are regulated independently of each other and that each cell type exert separate, but complementary, effects on TGF- β 1 fibrogenesis.

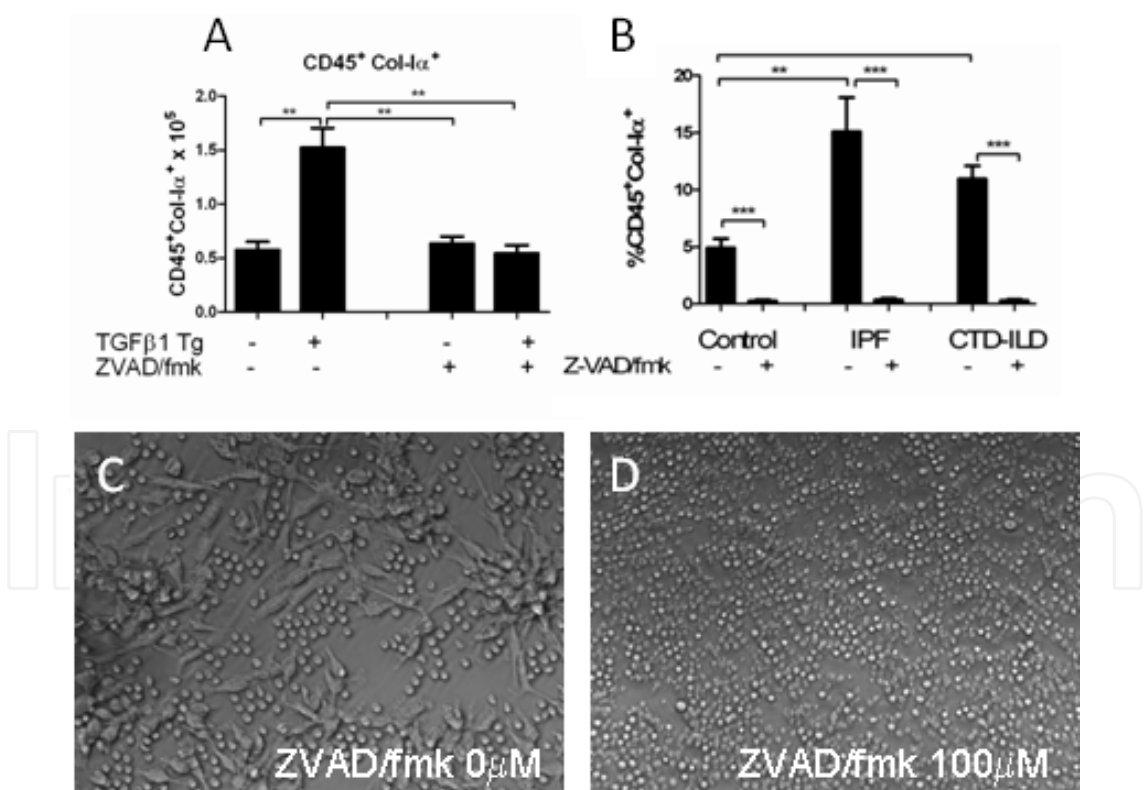


Fig. 2. Caspase inhibition administration attenuates apoptosis and collagen production in cultured human monocytes. (a) Z-VAD/fmk reduces CD45⁺Col-I α ⁺ cells in TGF- β 1 Tg⁺ mice. (b) Treatment with ZVAD/fmk attenuates collagen production in samples from all groups. (c,d) CT-ILD cultured monocytes fail to adopt a spindle shaped phenotype when treated with Z-VAD/fmk. (Peng et al., 2011)

4.4 Role of semaphorin 7a in fibrocyte outgrowth

Previous studies have indicated that fibrocyte biology is induced by exposure to soluble factors such as Th2 cytokines and activation of several chemokine pathways including CXCL12, MCP-1, and CCL21. However, the TGF- β 1 specific factors controlling their differentiation has remained less clear. Because SSc-ILD is associated with a unique “signature” of TGF- β 1 responsiveness (Sargent et al., 2010) we explored the role of TGF- β 1 signaling in fibrocyte accumulation. Because the monocyte-driven effects on fibrosis appear to be SMAD2/3 independent (Murray et al., 2011), we explored noncanonical TGF- β 1 pathways in this model. One such pathway is controlled by activation of Semaphorin 7a.

Semaphorins (Semas) are a family of highly conserved, secreted or membrane-associated proteins, expressed on stroma as well as nerve, myeloid and lymphoid cells. Originally discovered as axonal guidance proteins (Pasterkamp et al., 2003), eight classes of Semaphorins have since been discovered with Semas 3-7 subsequently being found to participate in a variety of processes related to organogenesis, angiogenesis, apoptosis, neoplasia and immune regulation (Pasterkamp & Kolodkin, 2003). Semaphorin 7a (Sema 7a), also called CDw108, is a GPI-anchored membrane protein that by signaling through its two main receptors, Plexin C1 and β 1 integrin, contributes to inflammation (Suzuki et al., 2007), modulation of T cell function (Czopik et al., 2006), and TGF- β 1 induced pulmonary fibrosis (Kang et al., 2007). However, until recently a role for Sema 7a in fibrocyte development had not been explored.

In order to explore this question we crossed TGF- β 1 mice with mice harboring null mutations of the Sema 7a gene. In addition to effects on lung fibrosis and remodeling, which had been described previously, we found that fibrosis (quantified via sircol analysis) and fibrocytes were markedly reduced in the TGF- β 1 mice that lacked Sema 7a (Figure 3a, $p < 0.001$). These effects were explored further in bone marrow chimera experiments in which TGF- β 1 mice were created in which Sema 7a expression was restricted to lung stroma or to bone marrow derived cells. Lung restricted Sema 7a expression in TGF- β 1 mice revealed a modest but insignificant reduction in fibrosis and fibrocyte content. However in the TGF- β 1 x Sema 7a null cohort of mice where Sema 7a expression was restored on bone marrow derived cells these mice developed increased fibrosis and fibrocytes (Gan et al., 2011). These studies reveal that Sema 7a expressing bone marrow derived cells are sufficient, but not necessary, for the development of fibrosis and fibrocyte accumulation in the TGF- β 1 exposed murine lung.

In order to explore the human relevance of these findings we interrogated the relationship between Sema 7a and fibrocyte biology in a second cohort of patients with SSc-ILD. In these studies, enhanced expression of Sema 7a and its two known receptors (β 1 integrin and Plexin C1) were detected in peripheral blood mononuclear cells (PBMCs) from patients with SSc-ILD, but not SSc only, indicating that the Sema 7a axis may be unique to patients with interstitial lung involvement. Flow cytometric analysis revealed that the increased expression of Sema 7a appeared to be related to augmented cell surface on fibrocytes and CD19+ lymphocytes. In contrast, Sema 7a receptors β 1 integrin and Plexin C1 were located on CD14+ monocytes (Gan et al., 2011). This led to speculation that exogenous ligation of these receptors by Sema 7a (either membrane bound or secreted) controls the monocyte to fibrocyte transition in this patient population.

The validity of this hypothesis was then tested in ex vivo studies of fibrocytes using standard, serum containing conditions. Specifically, monocytes from scleroderma subjects or controls were cultured in the presence or absence of recombinant Sema 7a stimulation for 14 days. These cultures were further exposed to Sema 7a receptor blockade of β 1 integrin or Plexin C1. In normal controls, Sema 7a exposure led to enhanced fibrocyte outgrowth, however, in

monocytes obtained from individuals with SSc-ILD, stimulation with Sema 7a had little effect on the already markedly enhanced fibrocyte numbers that exist at baseline in these cultures. In both groups these results were attenuated by $\beta 1$ integrin blockade and enhanced by Plexin C1 blockade (Figure 3 c,d). When viewed in combination it appears that Sema 7a controls the monocyte to fibrocyte transition in a $\beta 1$ integrin dependent manner that is opposed by Plexin C1 (Gan et al., 2011). Thus, the increased Plexin C1 gene expression seen in the SSc-ILD patients may represent a novel counter-regulatory response and a new target for therapy.

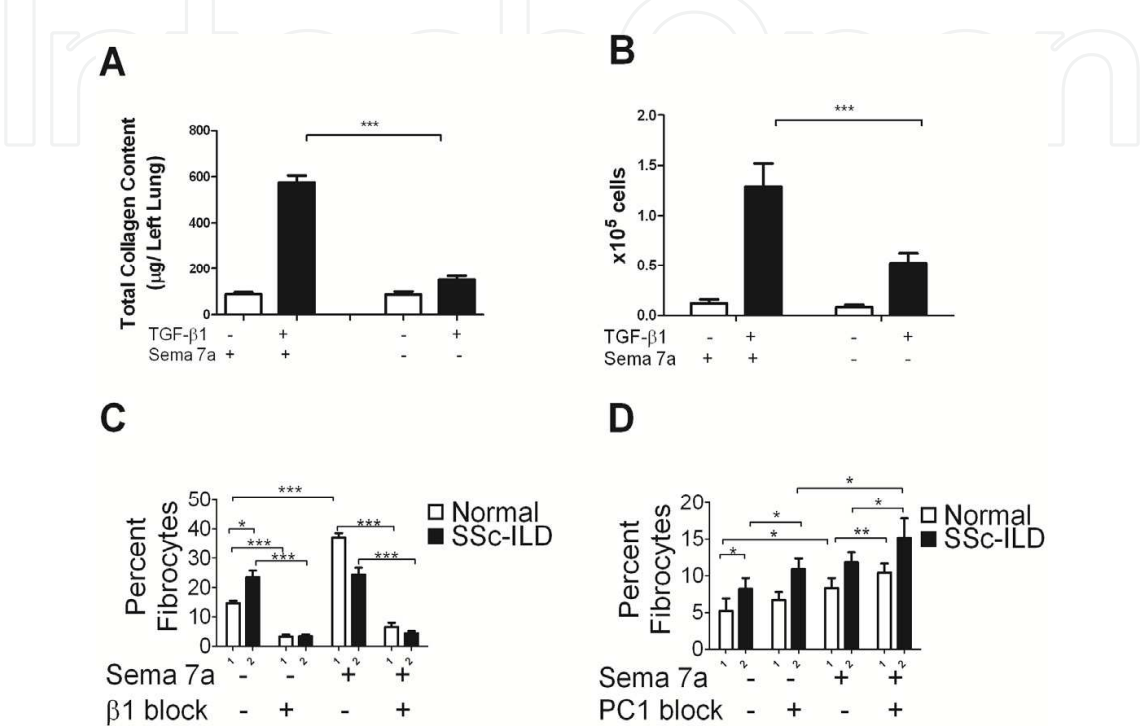


Fig. 3. (a) Total left lung collagen in wild type mice (white bar) and TGF- $\beta 1$ Tg⁺ mice (black bar). Left: Sema-7a locus intact. Right: Sema-7a locus deleted. (b) Compared to TGF- $\beta 1$ WT mice, lungs from TGF- $\beta 1$ X Sema-7a null mice contain significantly decreased quantities of CD45⁺Col-I α ⁺ cells. White bar: WT. Black bar: TGF- $\beta 1$ Tg⁺ mouse. (c) Sema-7a stimulated PBMCs show increased fibrocyte differentiation (white, left comparisons). In contrast, SSc-ILD subjects show increased fibrocyte outgrowth at baseline (black, left comparisons) with no response to exogenous Sema stimulation but pronounced reduction via immunoneutralization of the $\beta 1$ integrin subunit (right comparisons). (d) Inhibition of Plexin C1 promotes fibrocyte differentiation in Sema-7a stimulated monocytes from control and SSc-ILD subjects as well as in unstimulated SSc-ILD monocytes. (Gan et al., 2011)

5. Conclusion

Fibrocytes have been implicated in a wide array of human autoimmune and inflammatory diseases since their identification 17 years ago. Human and animal studies have confirmed elevated levels of these mesenchymal progenitor cells in blood and tissue during fibrotic disease states. Animal models have also revealed the fibrocytes' complex role in the repair and remodeling of injured tissue, which includes antigen presentation, ECM and cytokine production, promotion of angiogenesis and differentiation to myofibroblasts. Because of their role in the maintenance and homeostasis of connective tissue, they have emerged as a cell of interest in scleroderma, and specifically SSc-ILD. Our work in these patients revealed

a significant association between circulating fibrocytes and SSc-ILD. Targeting these cells, and their relationship with such regulatory factors as TGF- β 1, apoptosis, and Semaphorin 7a may ultimately lead to the discovery of new biomarkers and perhaps even novel therapeutic targets in SSc-ILD.

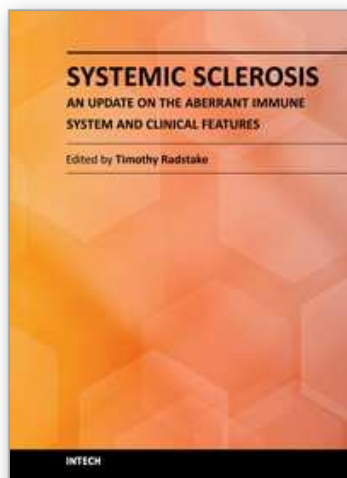
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Systemic sclerosis (SSc), or often referred to as Scleroderma (tight skin), is characterized by an exaggerated formation of collagen fibers in the skin, which leads to fibrosis. Accumulating evidence now points toward three pathological hallmarks that are implicated in Ssc, the order of which has yet to be determined: endothelial dysfunction, autoantibody formation, and activation of fibroblasts. This current book provides up-to-date information on the pathogenesis and clinical features of this severe syndrome. It is our hope that this book will aid both clinicians and researchers in dealing with patients with this clinical syndrome. In addition, we hope to shed more light on this rare and severely disabling syndrome, ultimately leading to better research and successful therapeutic targeting.

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